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RICHARD WARNER LINTON, A.M.
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MOBILIZATION AND TRANSFER OF CLASMATOCYTES *

RICHARD WARNER LINTON, A.M.
NEW YORK

The idea that mononuclear cells, the term being used in its broadest sense, are concerned in immunity to streptococcic infections is one that has been present in the literature for a long time. It is only in recent years, however, that attention has been focused on this relationship and that a tendency has been apparent to consider the mononuclears rather than the polymorphonuclears as the body's defenses in infections due to the streptococcus.

The question of the connection between the chief types of mononuclears, namely, the monocytes of the blood and the clasmatocytes or histiocytes of the tissues and the serous cavities, is one on which much work has been done but on which a final decision has not been reached. Simpson [1] believed that the identity of the two has not been proved, although there is no doubt of their close relationship. That the monocytes of the blood may change into macrophages in vitro has been shown by Lewis and Lewis.[2] On the other hand, Sabin and Doan [3] found both monocytes and clasmatocytes normally in the blood. Carrel and Ebeling [4] demonstrated that macrophages of the tissue and monocytes of the blood, when kept under similar conditions in tissue cultures, become identical. They believed that the two forms are different functional states of the same cell.

The cells designated variously as tissue macrophages, histiocytes, rhagiocrine cells, or clasmatocytes are the ones chiefly concerned in the present study. These cells offer the advantage of being easily recognized in the tissues through their reaction to certain vital dyes, notably trypan blue. They thus form part of the reticulo-endothelial system, as defined by Aschoff [5] and Kiyono.[6]

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* From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

1. Simpson, M. E.: J. M. Research **43**:77, 1922.
2. Lewis, W. H., and Lewis, M. R.: Transformation of White Blood Cells into Clasmatocytes, J. A. M. A. **84**:798 (March 14) 1925.
3. Sabin, F. R., and Doan, C. A.: J. Exper. Med. **43**:823, 1926.
4. Carrel, A., and Ebeling, A. H.: J. Exper. Med. **44**:285, 1926.
5. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, 1924.
6. Kiyono, K.: Die Vitalekarminspeicherung, Jena, Fischer, 1914.

The origin of these cells in lesions is probably double: from the monocytes of the blood, by change of function, and from the cells already present in the connective tissues. Lang [7] was unable to confirm the conclusion of McJunkin [8] and of Foot,[9] originally arrived at by Mallory,[10] that the capillary endothelium has a part in the formation of macrophages in the tissues; and in tissue cultures, Maximow [11] showed that endothelium gives rise to typical fibroblasts but not to ameboid elements. The connotation in the term "endothelial leukocyte" may thus be incorrect; by whatever term designated, however, the cells themselves are undoubtedly identical with clasmatocytes. Lewis [12] believed that the monocytes of the blood alone are capable of hypertrophying and forming macrophages and that the lymphocytes are not transformed into these cells. On the other hand, Maximow [13] stated that, in vitro, small lymphocytes change first into macrophages and then into fibroblasts.

Erysipelas was apparently the first streptococcic infection in which macrophages were observed. Metchnikoff [14] found large numbers of these cells in the lesions of human erysipelas, but asserted that the active resistance was due to the microphages. He believed that the function of the former cells was to take up weakened or dead polymorphonuclears, rather than bacteria. Later, Salimbeni,[15] working under Metchnikoff's direction on lesions of the skin in horses immunized against the streptococcus, noted the presence of macrophages in the tissues of the diseased areas, but attributed the healing process to the intervention of the polymorphonuclear cells. The predominance of small mononuclear cells in erysipelatous lesions has been further emphasized by MacCallum.[16]

A similar relationship has been found for other types of streptococcic infection. Thus, Bordet,[17] Zangemeister and Gans,[18] Wallgren,[19]

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7. Lang, F. J.: Rôle of Endothelium in Production of Polyblasts in Inflammation, Arch. Path. **1**:41 (Jan.) 1926.

8. McJunkin, F. A.: Am. J. Anat. **25**:27, 1919.

9. Foot, N. C.: J. M. Research **40**:353, 1919.

10. Mallory, F. B.: J. Exper. Med. **3**:611, 1898.

11. Maximow, A.: Klin. Wchnschr. **4**:1486, 1925.

12. Lewis, M. R.: Am. J. Path. **1**:91, 1925.

13. Maximow, A.: Proc. Soc. Exper. Biol. & Med. **24**:570, 1927.

14. Metchnikoff, E.: Virchows Arch. f. path. Anat. **107**:209, 1887.

15. Salimbeni, A. I.: Ann. de l'Inst. Pasteur **12**:192, 1898.

16. MacCallum, W. G.: Textbook of Pathology, ed. 2, Philadelphia, W. B. Saunders Co., 1920, p. 52.

17. Bordet, J.: Ann. de l'Inst. Pasteur **11**:177, 1897.

18. Zangemeister, W., and Gans, H.: München. med. Wchnschr. **52**:793 and 858, 1909.

19. Wallgren, A.: Beitr. z. path. Anat. u. z. allg. Path. **25**:206, 1899.

and Kanai [20] demonstrated that the chief cells taking part in the destruction of streptococci, both in the blood stream and in the peritoneum, are large mononuclears. More recently, Bass,[21] working on rabbits immunized by living streptococci injected intravenously, came to the conclusion that the immunity which his animals developed depends chiefly on the phagocytosis and digestion of the cocci by cells of the reticuloendothelial system (histiocytes, tissue macrophages and clasmatocytes). From further observations, he concluded that cells of the tissue macrophage type react to opsonized organisms as do the leukocytes. Cecil [22] and Rosenow and Ashby [23] showed the importance of macrophage cells in lesions of the joints and myocardial lesions due to streptococci, and the rôle of the fixed macrophages of the tissues has been emphasized in the studies of Hopkins and Parker,[24] Keyes [25] and Nagao.[26]

A great deal of information on the correlation between streptococcic infections and cells of the clasmatocyte type has been brought out by the researches of Gay with various collaborators. He showed [27] that by the injection of various substances into the pleural cavities of rabbits two types of exudate can be produced. Infusion broth or 1 per cent egg white calls out large numbers of clasmatocytes in twenty-four hours, while an exudate induced by whole egg white or aleuronat is predominantly polymorphonuclear in character at the same time interval. It was further shown that animals with pleural exudates of the first type are protected against many times the fatal dose of streptococci injected intrapleurally, while animals with large numbers of polymorphonuclear cells succumb to a fatal infection as readily or even more quickly than normal animals. Active immunization of the pleural cavity against streptococci is simultaneous with a definite increase in the number of clasmatocytes. It was also found that in cavities "prepared" by broth and infected, sterilization is coincident with a marked increase in the number of these cells.

The relationship between clasmatocytes and immunity to the streptococcus was further investigated in a study of the changes occurring in the tissues surrounding the pleural cavity.[28] It was shown here that

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20. Kanai, S.: Verhandl. d. Japan, path. Gesell. **1**:126, 1919.

21. Bass, F.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **43**:269, 1925.

22. Cecil, R. H.: J. Exper. Med. **24**:739, 1916.

23. Rosenow, E. C., and Ashby, W.: Focal Infection and Elective Localization in Etiology of Myositis, Arch. Int. Med. **28**:274 (Sept.) 1921.

24. Hopkins, J. G., and Parker, J. T.: J. Exper. Med. **27**:1, 1918.

25. Keys, P.: J. Infect. Dis. **18**:277, 1916.

26. Nagao, K.: J. Infect. Dis. **27**:327, 1920.

27. Gay, F. P., and Morrison, L. F.: J. Infect. Dis. **33**:338, 1923.

28. Gay, F. P.; Clark, A. R., and Linton, R. W.: Histologic Basis for Local Resistance and Immunity to Streptococcus, Arch. Path. **1**:857 (June) 1926.

the character of the exudate reflects the condition of the subjacent tissues. A cavity, prepared seventy-two hours before infection by the injection of a mixture of gum arabic and beef extract, quickly becomes sterile; its walls are found to be thickened and filled with a granulation tissue. Many of the cells of this tissue are clasmatocytes, and in them organisms could be observed undergoing destruction. On the other hand, when the pleural walls have been acutely inflamed by the injection of aleuronat and starch eighteen hours previously and when they are filled with polymorphonuclear cells almost exclusively, the animals are without protection. The organisms quickly invade the inflamed areas; necrosis sets in, and death of the animal follows in four or five days. The phagocytosis of streptococci by polymorphonuclears is negligible in these tissues.

It thus appears from the work of Gay and his collaborators that there is a definite and constant correlation between clasmatocytes and resistance to streptococcic infections in the pleural cavities of rabbits.

The importance of macrophages of the tissues (clasmatocytes) in other bacterial infections has been emphasized by various workers. Thus, Buxton [29] concluded that the resistance of rabbits immunized against typhoid is dependent on the increased ability of the macrophages to destroy the organisms, and Jelin [30] showed that the blood of rabbits is rid of *B. typhosus* by means of cells of the macrophage type. By means of vital staining, Oliver [31] demonstrated that the lepra cell in leprosy in rats is derived from the histiocyte. A monocytosis in malaria, typhus fever and relapsing fever was reported by Kartaschawa [32] and in cholera by Netousek.[33] In kala-azar, Meleney [34] found that the body reacts to the parasite by the proliferation of the large mononuclear phagocytes of the connective tissues. These observations have been extended by Hu and Cash,[35] who stated that the typical lesions of this disease contain cells of the reticulo-endothelial system almost exclusively. It was further shown that this system is practically the only part of the body involved in the disease. Jungeblut [36] further emphasized the importance of a normal reticulo-endothelial system in protozoan infections and has suggested that chemotherapeutic substances which are inactive in vitro may owe their activity in vivo to the cells of this system.

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29. Buxton, B. N.: J. M. Research **16**:251, 1907.
30. Jelin, W.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **47**:199, 1926.
31. Oliver, J.: J. Exper. Med. **43**:233, 1926.
32. Kartaschawa, F. W.: Arch. f. klin. Med. **146**:226, 1925.
33. Netousek, M.: Folia haemat. **17**:407, 1913.
34. Meleney, H. E.: Am. J. Path. **1**:147, 1925.
35. Hu, C. H., and Cash, J. R.: Proc. Soc. Exper. Biol. & Med. **24**:469, 1927.
36. Jungeblut, C. W.: Ztschr. f. Hyg. u. Infektionkrankh. **107**:357, 1927.

More closely related to the work to be reported in the present paper are the results of Nakahara.[37] This author induced a macrophage reaction in the peritoneal cavity of mice by injecting olive oil. Coincident with this increase in macrophages, there occurs a local resistance to fatal doses of staphylococci and pneumococci as well as a quicker disposal of *Bacillus coli*. The resistance is due to phagocytosis of the organisms by the macrophages. When *Bacillus coli* is injected into the pleural cavities, however, it does not disappear any more rapidly in animals with a peritoneal exudate of macrophages than in the normal controls.

My purpose in the present study has been to determine the relationship between a focus of cells of known type in one part of an animal's body and an irritant, either animate or inanimate, in another part. Interest in this problem came from work reported elsewhere (Gay, Linton and Clark[38]) on the relations of immunity between the two pleural cavities. In these papers it was shown that the immunization of one cavity against a streptococcic infection, accomplished by the formation in its wall of a granulating tissue rich in clasmatocytes, is coincident with the immunization of the other cavity. The two cavities, while equally protected, differ histologically in that the untreated one is normal in appearance before infection. After it is irritated, however, large numbers of clasmatocytes quickly appear in its walls, and by means of vital staining it could be shown that at least part of these cells came from the focus on the opposite side.

In the present work the relationship between a focus of cells in the peritoneal cavity and irritants or streptococci in the pleural cavities has been particularly studied. The right cavity has always been used for the tests. The left cavity has served as a control in some of the experiments. The first part of the work deals with experiments in which the focus of cells was formed in the peritoneum, followed by the injection of aleuronat and starch in the right pleural cavity. In the second half of the work, streptococci were used as the intrapleural irritant, subsequent to the formation of a peritoneal focus of cells.

Rabbits weighing about 2,000 Gm. were used. Tissue was fixed in Zenker's fluid without acetic acid. Sections were cut at 8 microns and were stained with eosin and methylene blue, and with safranine alone as a counter stain for the trypan blue. The pieces used for embedding and sectioning were taken as nearly as possible from the same area in each animal, namely, between the sixth and seventh ribs in the pleural wall, and to the right of the midline and somewhat laterally in the peritoneum.

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37. Nakahara, W.: J. Exper. Med. **42**:201, 1925.

38. Gay, F. P.; Linton, R. W., and Clark, A. R.: Proc. Soc. Exper. Biol. & Med. **24**:23, 1926; (footnote 28).

A part of the omentum was taken in many cases. It was prepared by being spread over a slide, allowed to dry in air, fixed for five minutes in absolute methyl alcohol, and finally stained with safranine or Wright's stain.

MOBILIZATION BY MEANS OF ALEURONAT AND STARCH

The following typical protocol illustrates the methods used in the first part of the work:

PROTOCOL A

Group A. 5 cc. of aleuronat and starch[39] injected intraperitoneally, first day
5 cc. of aleuronat and starch injected intraperitoneally, third day
5 cc. of aleuronat and starch injected intraperitoneally, fifth day
5 cc. of 1 per cent aqueous trypan blue[40] injected intraperitoneally, seventh day
3 cc. aleuronat and starch injected into the right pleural cavity, ninth day
Killed twelfth day

Controls:

Group B. 5 cc. of 1 per cent aqueous trypan blue injected intraperitoneally, seventh day
3 cc. aleuronat and starch injected into the right pleural cavity, ninth day
Killed twelfth day

Group C. 5 cc. of aleuronat and starch injected intraperitoneally, first day
5 cc. of aleuronat and starch injected intraperitoneally, third day
5 cc. of aleuronat and starch injected intraperitoneally, fifth day
5 cc. of 1 per cent aqueous trypan blue injected intraperitoneally, seventh day
Killed twelfth day

Group D. 5 cc. of 1 per cent aqueous trypan blue injected intraperitoneally, seventh day
Killed twelfth day

The three intraperitoneal injections of the aleuronat-starch mixture on alternate days were intended to irritate the serosal lining of this cavity and cause the formation of a granulating tissue, which would contain clasmatocytes. The presence of these cells was determined by the use of trypan blue. Histologically, the peritoneal walls of animals thus treated and killed on the twelfth day showed marked changes (fig. 1). The cells of the serosal lining were intact and swollen. The subserous changes were striking. Enormous numbers of clasmatocytes as well as numerous fibroblasts were present; the latter were distinguished from the former by the small amount of dye stored in their cytoplasm. Polymorphonuclears were relatively rare. New blood vessels had been

39. This is a mixture of 5 per cent aleuronat and 3 per cent starch.

40. The trypan blue (National Aniline & Chemical Works) is made up in distilled water, filtered and autoclaved for twenty minutes before use.

formed throughout, and in some sections bits of the irritant were found below the intact serosa. The thickness of the wall had been increased from approximately 30 microns to an average of 300 microns. The picture was thus that of a typical granulation tissue. The omentums from these cavities also showed a large increase in clasmatocytes.

The animals in group A were designated to test the possibility of "cell transfer" from the peritoneum to the right pleural cavity. Hence, a peritoneal focus of clasmatocytes was first formed by the injection of the aleuronat and starch mixture, and subsequently the right pleural cavity was irritated. The animals in the other three groups served in various ways to control the A group. Thus, animals in group B were

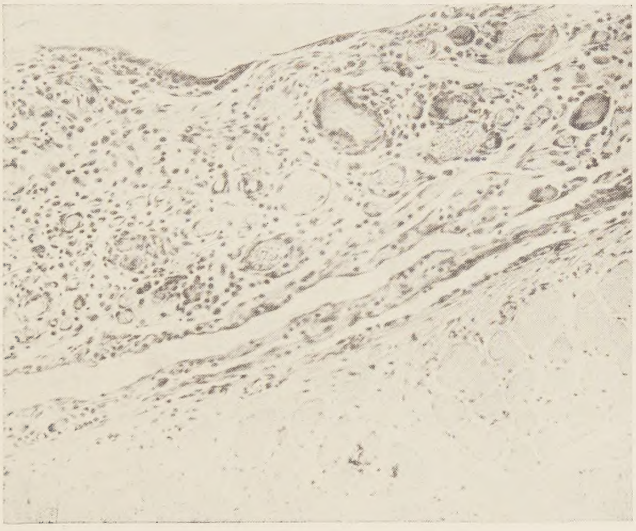

Fig. 1 (rabbit 5042).—Peritoneal serosa of a rabbit given three intraperitoneal injections of aleuronat-starch mixture. Large mass of granulation tissue, averaging 472 microns in width containing numerous clasmatocytes; × 120.

given dye in the peritoneum, which was otherwise untreated. They received the same amount of irritant in the pleural cavity and at the same time as the animals in group A and thus controlled the efficacy of the pleural irritant itself in causing changes in the serosae. Conversely, the animals in group C., in which dyed peritoneal foci were formed, tested the effect of these granulating areas on untreated pleural cavities. Group D, in which the animals were given a single intraperitoneal injection of dye, was designed to show the effect of trypan blue alone on the surfaces of the peritoneal and pleural cavities.

The character of the exudates, as well as their structure and composition, was studied since it had been shown elsewhere that it reflects the

condition of the underlying tissues. The results of these studies are shown in table 1. The most interesting contrast is that between groups A and B; that is, between animals with equally irritated pleural cavities, but differing in that the first contains a "peritoneal focus" of clasmatocytes. In respect to the amount of fluid in the right pleural cavity, the A group is slightly superior, about 3 cc. being present in comparison with 2 cc. in the animals that had not received peritoneal irritation; in the latter, however, the total number of cells is somewhat greater: from 254 to 211 million. But it is in the relationship between the cell types that the chief differences lie. In animals with merely irritated pleural cavities, the proportion between the average number of clasmatocytes and the average number of polymorphonuclears is 1 : 2.5, while in the group having a "peritoneal focus," the clasmatocytes are much higher,

TABLE 1.—*Summary of Studies of Exudates in the Right Pleural Cavities of Rabbits in Groups A, B, C and D*

	Amount Fluid, Cc.	Total Cells*	Total Clasmatocytes	Total Polymorphonuclears	Percentage Clasmatocytes	Percentage Polymorphonuclears	Percentage Trypan Blue	Total Trypan Blue Cells*
Group A (9)†...	3.2	211.0	129.3	81.6	60	40	4	1.3
Group B (5)....	2.1	254.0	74.0	180.0	30	70	10	7.5
Group C (3)....	0.1	0.4	0.35	0.048	88	12	0	0.0
Group D (3)....	0.01	0.28	0.25	0.019	92	8	11	0.034
Normal control	0.1	0.35	0.3	0.05	85	15	0	0.0

* In millions.
† The figures in parenthesis give the number of animals on which the averages are based.

the proportion being 1 : 0.5. Furthermore, there are about twice as many clasmatocytes in the right pleural cavities of animals in group A as in those in group B; that is, approximately 130 million in contrast to 74 million. If one expresses the values as percentages of the total white blood cells present in these exudates, animals without "peritoneal foci" show 30 per cent clasmatocytes, while animals with such granulating areas show 60 per cent clasmatocytes. In brief, the animals with a "peritoneal focus" of clasmatocytes show a decided superiority both in the relative and in the absolute numbers of these cells in their irritated pleural cavities.

The exudates of the peritoneal and left pleural cavities do not differ appreciably in the two groups, although here, too, the percentage of clasmatocytes is higher in the nonirritated left cavities of A than in B (80 per cent in contrast to 65 per cent). The exudates in group C are those of normal cavities. Accordingly, the peritoneal irritant itself does not affect the ratios or the totals of the cells in the pleurae, nor does the

peritoneal aggregation of cells cause any intrapleural changes. Likewise, in group D the dye did not change the proportion of cells to each other or their totals, and the exudates were normal.

HISTOLOGIC OBSERVATIONS

The normal serosal lining of the parietal and diaphragmatic pleurae, as well as that of the peritoneum, consists of a single layer of flat mesothelial cells. The subserosa, which lies between the mesothelial cells and the muscles, is formed of bundles of elastic fibers lying in various directions and interspersed with a few mononuclear cells, some of which by vital staining can be distinguished as tissue macrophages or clasmatocytes. Polymorphonuclear cells are rarely found.

When an irritant such as an aleuronat-starch mixture is injected into the pleural cavity, a typical inflammatory reaction develops in the serosal lining. The subserous and adjacent intramuscular capillaries become congested and from them large numbers of polymorphonuclear cells appear, and pass out into the cavity between the serosal cells. The latter are swollen and show some mitosis. Seventy-two hours after the injection of the irritant, the polymorphonuclears have practically disappeared from the subserosa; their place has been taken by macrophages and fibroblasts. In animals in which vital stains have been employed, many of the macrophages prove to be clasmatocytes. The serosal cells are rounded up and show much mitotic activity. In some cases they may form a layer two or three cells in thickness. In short, at this period, the acute inflammation has given way to a characteristically chronic condition with clasmatocytes as one of the chief components of the reaction.

The animals in groups A and B were killed seventy-two hours after the intrapleural injection of the irritant. The course of events already described was found to occur in both groups, and the character of the infiltration at this time in both was predominantly mononuclear. In this respect the presence of a large focus of clasmatocytes did not effect a difference in the two groups. The two groups do differ markedly, however, in the amount of thickening which their walls have undergone. This thickening is, in general, proportional to the number of cells which have infiltrated the area, but while it is due chiefly to cellular infiltration, two other factors play a part: the accumulation in the tissue of fluid, both in the lymph spaces and between the cells, and an increase in the number of connective tissue fibers. The number of mononuclears in the walls, as well as the amount of intercellular fluid, is generally greater in animals with a "peritoneal focus" than in animals with normal peritoneums. This fact also finds expression in the amount and character of the exudates in the two groups. As already pointed out, the amount

of pleural fluid in group A is about one-half larger than that in group B, while the number of clasmatocytes is twice as great. On the other hand, the fibers of the connective tissue vary irregularly. In some instances, they do not show any increase in number; in other animals, their number is somewhat larger than normal. There does not seem to be any constant relationship between them and the presence or absence of a peritoneal focus. In no case, however, is their variation from normal striking.

Table 2 gives the measurements, in microns, of the serosal linings of the various groups.[41] Each figure represents the average of several determinations of the distance from the muscles to the outer margin of the serosal cells. The right pleural wall of group A averages 177 microns or about six times the normal width, while the corresponding measurement in group B gives approximately four times the normal thickness. The most striking contrast, however, occurs in the pleural diaphragms of the two right cavities. Group B in this tissue shows a

TABLE 2.—*Summary of Measurements of Serosal Linings, in Microns*

	Peritoneal Wall	Right Pleural Wall	Right Diaphragm		Left Pleural Wall	Left Diaphragm	
			Peritoneal Wall	Pleural Cavity		Peritoneal Wall	Pleural Cavity
Group A.....	414	177	57	494	67	49	47
Group B.....	33	122	45	210	30	41	32
Group C.....	367	34	58	34	..	..	..
Group D.....	47	25	38	41	..	..	..
Normal control..	32	28	36	28	28	36	28

thickening of seven times normal, while the animals with a "peritoneal focus" (group A) show a serosal lining seventeen times normal in width.

The left cavities of these two groups show a smaller but still a clear-cut difference. Histologically, the superior thickening of the left cavities of the animals in group A is due entirely to an infiltration of monocytes. This change has not occurred in the other group in which the walls have remained normal.

The determinations in group C show that without irritation an effect is not produced in the right pleural wall or diaphragm of animals with a peritoneal focus. Similarly, the measurements of animals in group D are approximately normal throughout. In the last two groups the left cavities were not studied histologically, as it was assumed that they would not differ appreciably from the right cavities.

41. The measurements were made by means of a filar micrometer. Points above the trabeculae of the connective tissue were always avoided in making the determinations.

As would be expected from the figures, the cellular response to the irritant is found to be stronger histologically in the pleural diaphragm than in the pleural wall. This difference is particularly marked in group A. In the diaphragm, cords of mononuclears extend from the pleural surface down between the muscle bundles. In some instances this collection of monocytes in the tissues is marked as far down as the middle of the diaphragm. Many of these cells contain trypan blue; there does not seem to be a constant relationship between them and the capillaries. This intermuscular collection of mononuclears and clasmatocytes is present only to a slight degree in group B. Here the infiltrating cells tend to collect under the pleural serosa and extend into the muscles only a short distance. This difference in appearance and in the number of cells present does not come out in the serosal measurements, since distances below the muscles are not measured. A similar intermuscular collection of cells, also presumably from a distant focus, has been reported in transfer experiments between the right and left cavities (Gay, Clark and Linton [28]).

The parietal pleurae of the two groups resemble each other in general, with the exception of the number of cells which has appeared in each. Intermuscular cords of mononuclears are absent in both, although the trabeculae of the connective tissue may be crowded with cells for a short distance below the surface. The pleural surfaces of the wall and diaphragm are much more alike in group B than in group A.

Thus, under the conditions of these experiments, animals with "peritoneal foci" show in their irritated pleural cavities a marked superiority both in the number and proportion of clasmatocytes present over controls without such foci. Similarly, the serosal linings of their pleural walls and diaphragms show a decided increase in thickening in contrast to the controls. This thickening is due principally to infiltration by clasmatocytes. The distribution of cells containing trypan blue in these pleural cavities will be discussed later.

MOBILIZATION BY MEANS OF INTRAPLEURAL STREPTOCOCCIC INFECTION

The following protocols were used in this part of the work:

PROTOCOL B

Group 1. 5 cc. aleuronat and starch injected intraperitoneally, first day
5 cc. aleuronat and starch injected intraperitoneally, third day
5 cc. aleuronat and starch injected intraperitoneally, fifth day
From 100 to 250 times the minimum lethal dose of a twenty-four hour broth culture of passage fluid streptococcus "H" in 1 cc. amount injected intrapleurally into the right cavity, ninth day

Group 2. 5 cc. aleuronat and starch injected intraperitoneally, first day
5 cc. aleuronat and starch injected intraperitoneally, third day
5 cc. aleuronat and starch injected intraperitoneally, fifth day
5 cc. of 1 per cent aqueous trypan blue injected intraperitoneally, seventh day
From 100 to 250 times the minimum lethal dose of a twenty-four hour broth culture of passage fluid streptococcus "H" in 1 cc. amount injected intrapleurally into the right cavity, ninth day

Group 3. From 100 to 250 times the minimum lethal dose of a twenty-four hour broth culture of passage fluid streptococcus "H" injected intrapleurally into the right cavity, ninth day

Group 4. 5 cc. aleuronat and starch injected intraperitoneally, first day
5 cc. aleuronat and starch injected intraperitoneally, third day
5 cc. aleuronat and starch injected intraperitoneally, fifth day
5 cc. of 1 per cent aqueous trypan blue injected intraperitoneally, seventh day
Killed ninth day

In groups 1 and 2, the same methods for producing peritoneal granulating areas which had proved successful in the work already reported were employed.

The animals in group 1 differ only in that they were not given trypan blue intraperitoneally. Group 3 indicated the course of the intrapleural infection in normal animals. The rabbits in group 4 presented the condition of the pleural cavities at the time of infection; these animals had dyed "clasmatocyte foci" in their peritoneums, while their pleural cavities were untouched. They were killed at the same time that the animals of groups 1 and 2 were infected. The strain of streptococci used here is the same one which has been repeatedly described by Gay and his co-workers.[27] It was originally derived from a fatal human case of empyema, and its virulence for rabbits has been raised to a high degree by repeated passages through the pleural cavities of these animals at intervals of about one month. Between passages, it is kept in pleural fluid in the icebox. Approximately fifteen chains now constitute a minimal lethal dose. The accurate method of counting which has been developed is described elsewhere.[27]

Of the twenty-six animals infected and allowed to go on to death or recovery, twelve belonged to group 1 and fourteen to group 2. Of the entire number, seven animals, or 27 per cent died with typical pleural infections at the usual interval of four or five days. The controls in group 3 always died with typical infections. Intraperitoneal preparation thus gives a survival of 73 per cent of the infected animals. It is interesting to note that four of the seven unprotected animals had been treated with dye (group 2), while among the nineteen survivors only six had been so treated. If one expresses the values in percentages, 31 per

cent of the surviving animals had been given trypan blue, and 57 per cent of the unprotected animals were thus treated. These figures seem to indicate that the addition of the dye decreases the animal's chance of surviving by about one-half. Since it is clear that "clasmatocyte transfer" is as good in animals given dye as in those not receiving it, it appears that the dye in some way interferes with the functioning of the cells in the pleural cavity.

Since a more detailed study of the process of clearing up was desirable and since a preliminary experiment had shown that the critical period in the sterilization of these animals lay between forty-eight and seventy-two hours after infection, a number of rabbits were treated similarly to those in group 2 and killed at intervals of from twenty-four

TABLE 3.—*Summary of the Study of Exudates of the Right Pleural Cavities of Rabbits with a Peritoneal Focus of Clasmatocytes, Killed at Intervals After Infection in the Right Pleural Cavity with from 100 to 260 Times the Minimum Lethal Dose of Passage Fluid Streptococcus "H"*

	Number Organisms Given	Number Organisms Recovered per Cc.*	Amount of Fluid, Cc.	Total Cells*	Total Clasmatocytes	Total Polymorphonuclears	Percentage Clasmatocytes	Percentage Polymorphonuclears	Percentage with Streptococci: Clasmatocytes	Percentage with Streptococci: Polymorphonuclears	Trypan Blue Cells: Percentage	Trypan Blue Cells: Number
Normal.....	.....	...	0.1	0.35	0.3	0.05	85	15	..	..	..	..
Control† (3)...	.....	...	0.1	0.3	0.25	0.05	83	17	..	..	..	..
24 hour (7)....	3,400	127	5.2	359.0	17.0	342.0	8	92	0	3.7	0	0
48 hour (7)....	3,000	1.9	3.1	267.0	35.0	232.0	21	79	2	0	0	0
72 hour (8)....	3,000	Sterile	0.13	4.1	3.4	0.7	80	20	0	0	2	0.07
5099	4,400	Sterile	1.8	4.3	1.1	3.1	27	73	0	0	2	0.022

* In millions.
† Animals with peritoneal foci, killed without intrapleural infection.

to seventy-two hours. A less intensive study was made of animals between the three day and two week periods. A single animal was studied fifty days after the infection.

Table 3 shows the studies of the exudate of the infected cavities. In animals with a "peritoneal focus" that were killed without infection being produced, it was found that the presence of a large intraperitoneal focus of clasmatocytes did not have any effect on the pleural cavities either in regard to the amount of fluid contained in them, the number or kind of cells present or the character of the walls. In all these respects, they were normal at the time the streptococci were injected. These cavities contained 0.1 cc. of fluid, in which were found about 350,000 white cells. Of these, approximately 300,000, or 85 per cent, were clasmatocytes; the other 15 per cent were polymorphonuclears. Furthermore, clasmatocytes showing dye were not present in their walls or in their pleural exudates, and their subserous tissues were normal in thickness.

TWENTY-FOUR HOUR PERIOD

Between twenty-four and thirty-three hours after the administration of the test dose (from 100 to 250 times the minimum lethal dose), the character of the exudate had markedly changed. It averaged[42] 5.2 cc. of turbid fluid. The organisms had multiplied enormously, more than 40,000 times the number injected being recovered in the platings from the right cavity. The changes in the cells were no less marked. Both clasmatocytes and polymorphonuclears showed great, though unequal increases. Of the 359 million cells present, 342 million of them (92 per cent) were polymorphonuclears. The clasmatocytes, originally composing 85 per cent of the cells present, now were only 8 per cent of the total, although their absolute number was large (17 million). The proportion between the clasmatocytes and the polymorphonuclears was 1 : 20. Phagocytosis occurred only in polymorphonuclears and in only a small proportion of these cells (from 3 to 4 per cent). Organisms did not appear in the clasmatocytes, and cells with trypan blue were not to be found.

Thus, at this period an acute condition with enormous numbers of polymorphonuclear cells, extensive proliferation of the injected organisms and only a slight manifestation of any resistance on the part of the infected animal occurred.

Normal animals reacted to a fatal intrapleural dose of streptococci in twenty-four hours by the production of an exudate which is also composed of about 90 per cent polymorphonuclears. The total number of cells produced, however, was only about one fifth as great, and the number of clasmatocytes was one-third as high as in the animals with a "peritoneal focus" (Gay and Morrison[27]).

Histologically, the protected animals showed pleural walls with an acute inflammatory condition. Their width was increased, on the average, to 136 microns; it was due largely to an infiltration of polymorphonuclear cells and in small part to an increase in the number of monocytes. As was shown in the previous part of the work, the diaphragmatic surface under these conditions reacted to irritants more strongly than the parietal surface. At this period it was thickened to an average of 252 microns and was covered with a seropurulent exudate in which masses of bacteria were present. In both tissues the most striking feature was the amount of diapedesis of the polymorphonuclears, both from the capillaries and from the subserosa into the cavity. It is important to note that dyed cells were not to be found on the wall in any case, although the diaphragm showed a few. Fibrin was not present in the exudate or under the serosa.

The distribution of organisms was as follows: They were visible in large numbers in the seropurulent exudate along the wall and along the diaphragm, and in some places lay in tight masses against the serosal cells. None was found within these cells. In two of the animals a few free chains of streptococci were present below the serosa, the cells of which were swollen and drawn apart. Since a particular reaction did not surround these organisms, it is probable that their invasion had been a recent one.

The acute inflammatory condition of the exudate was thus reflected in the walls.

Twenty-four hours after the administration of the test dose, only relatively slight changes had occurred in the serosae of normal animals given a fatal dose.

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42. In the averages made at this period, the single animal which was found sterile (5099) is not included.

These changes were of the same kind, although far less marked, as those in animals with a "peritoneal focus"; namely, a polymorphonuclear infiltration, seropurulent exudate and swelling of the serosal cells.

A single animal (5099) was found sterile at this period (table 3). The possibility of a faulty inoculation in this case was ruled out by the cell counts. The number of cells in the cavity as well as the amount of fluid was relatively small, while the proportion of clasmatocytes (27 per cent) was much higher than in animals which had not become sterile. It is significant that in this animal alone, at this period, dyed cells appeared in the exudate, to the amount of 2 per cent of the clasmatocytes.

The histologic manifestations in this animal were as follows: the reaction was marked along the diaphragm and less so on the pleural wall. The polymorphonuclears in either situation were so few as to be negligible. The reaction was a mononuclear one and in the diaphragm consisted practically entirely of dyed clasmatocytes. This surface was 111 microns thick, while the wall averaged 80 microns. Dyed cells were not present in the latter situation. Organisms were not visible in either tissue. There seemed to be a striking correlation here between the clearing up of a streptococcic infection and the presence of clasmatocytes.

FORTY-EIGHT HOUR PERIOD

Forty-eight hours after the administration of the test dose (table 3), the average amount of fluid had dropped to 3.1 cc., and the number of organisms was about 1.9 million per cubic centimeter in contrast to the 127 million per cubic centimeter of the twenty-four hour period. In two of the animals killed at this period, the number of organisms was only about twice that injected. Of further significance is the fact that the clasmatocytes had doubled, while the polymorphonuclears were less than two-thirds as numerous as at the twenty-four hour period. Furthermore, the number of organisms was most reduced in animals which had the highest relative and absolute clasmatocyte counts. The average proportion between the two chief types of cells was now approximately 1 clasmatocyte to 6 polymorphonuclears. The only phagocytosis was confined to the clasmatocytes; polymorphonuclears were inert in this respect. Trypan blue cells were not found in any of the exudates in which they were sought.

The condition of the exudate at this period may be summed up by pointing out that there were increases in the number and proportion of clasmatocytes, with corresponding decreases in the polymorphonuclears and in the number of bacteria present. The average amount of fluid was also somewhat decreased. Phagocytosis by polymorphonuclears had ceased, and had begun in the clasmatocytes.

Normal animals given fatal intrapleural doses showed, after forty-eight hours, an increased amount of fluid over the twenty-four hour animals, about 12 cc. being present on the average. The cellular contents of this exudate was formed almost entirely of polymorphonuclears, the clasmatocytes amounting to less than 1 per cent of the total.

The histologic manifestations in the protected animals were interesting. The number of macrophages in the walls was greatly increased, and there was a corresponding diminution of polymorphonuclars, so that the average thickening of the wall remained about the same as at the twenty-four hour interval (115 microns). Large numbers of dyed clasmatocytes had entered the subserous tissues. Some of these contained cellular debris and polymorphonuclears. The streptococci were distributed as follows: most of them lay among the clasmatocytes in the

serous exudate above the intact serosa. They were also found within the serosal cells, and below them in the tissue, either free in the enlarged lymph spaces or within clasmatocytes. In some places, the serosal cells were swollen and drawn apart without giving rise to new cells to fill in the gaps thus formed. Hence, there was a portion of the wall left exposed and covered only by fibers of connective tissue. These places, however, had under them many fewer bacteria than were found under the swollen serosal cells. From this observation, it appears possible that the organisms entered the tissue by being taken up by the serosal cells, either actively or passively, and passed back by them into the subserosa where they were rephagocytosed by macrophages.[43] All stages in the digestion of cocci may easily be found in the subserous clasmatocytes at this period. The capillaries showed a monocytosis, but none of the monocytes in this position contained any trypan blue. A small number of clasmatocytes undergoing mitosis were present.

What has been said applies chiefly to the pleural wall. In the diaphragm, the acute condition of the twenty-four hour period persisted for a longer time. Here the polymorphonuclears were more numerous, and the serosa was broken and even stripped off in places. At this period in the single animal with an infected peritoneal cavity (5088), organisms could be found free throughout the diaphragm. Dyed cells were also scattered throughout but occurred chiefly at the pleural and peritoneal surfaces. Mitotic figures, particularly of serosal cells, were frequent.

The most striking histologic feature at this period is the simultaneous appearance of large numbers of clasmatocytes and of streptococci below the serosae of the infected areas.

Forty-eight hours after the administration of the test dose, the normal controls showed histologically an extension of the acute inflammation which was beginning at twenty-four hours. The serosa was necrotic and was covered with an irregular fibrinopurulent exudate. Mononuclear cells were not visible either above or below the serosa. The capillaries showed masses of polymorphonuclears but not any mononuclear cells. Numerous streptococci were scattered throughout the exudate, although there had not been any infiltration of organisms into the subserous tissues.

SEVENTY-TWO HOUR PERIOD

Seventy-two hours after the injection of organisms, five of the animals killed were found to have sterile right cavities. Of the other three, twenty organisms were found in one, and in the two others one colony was obtained on the 1:100 dilution plates. The amount of fluid present was below normal in all the animals except one. The total number of cells, while still considerably greater than normal, was greatly reduced in comparison with the number previously present. The change in cellular character was pronounced: 80 per cent of the 4 million cells were clasmatocytes, and the ratio between these cells and the polymorphonuclears had risen to 1:0.25. Phagocytosis was not found in the animals which were still infected, due, no doubt, to the small number of organisms remaining. In two of the animals, dyed cells had appeared in the cavities.

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43. Cunningham (Am. J. Physiol. **62**:253, 1922) showed that most particulate matter is removed from the peritoneum by being forced into the cytoplasm of the serosal cells through the movements of the diaphragm against the viscera; the amount carried into the subserosa by phagocytes is small.

At this period and up to death, which usually occurred after five days, normal animals showed only polymorphonuclears in their exudates, increasing numbers of which were undergoing disintegration. The amount of fluid increased steadily and some times amounted to 25 or 30 cc.

Histologically, these animals showed a deep deposit of fibrin above an entirely necrotic serosa. In the subserosa, necrotic changes were also apparent: polymorphonuclear cells and muscle bundles were undergoing a decided disintegration. Fibrin and plugs of streptococci had appeared in the subserous lymph spaces. More macrophages were present than on the previous days and seemed relatively unaffected by the general necrosis, but their number was small.

A decided contrast is furnished by the observations for the seventy-two hour "peritoneal focus" animals. The wall averaged 100 microns, which was slightly less than at the former periods. The tissue was rapidly returning to normal. The number of monocytes was somewhat reduced over the previous intervals, and a large proportion of them showed their clasmatocytic nature by the storage of trypan

TABLE 4.—*Cells in the Peritoneal and Left Pleural Cavities of Animals with a "Peritoneal Focus" Infected in the Right Pleural Cavity with 100 to 250 Times the Minimum Lethal Dose of Passage Fluid Streptococcus "H" and Killed at Intervals Thereafter*

	Peritoneum				Left Cavity				Heart's Blood:	
	Percentage	Percentage	Number of Animals with		Percentage	Percentage	Number of Animals with		Number of Animals with	
Time, Hours	Clasmatocytes	Polymorphonuclears	Infected Cavities	Sterile Cavities	Clasmatocytes	Polymorphonuclears	Infected Cavities	Sterile Cavities	Infected Cavities	Sterile Cavities
0	64	36	...	...	95	5	...	...	...	...
24	83	17	1	5	17	83	7	1	3	3
48	93	7	1	6	12	88	5	2	2	5
72	70	30	0	6	92	8	0	8	0	8

blue in their cytoplasm. Most of the few polymorphonuclears present were within macrophages. The serosal cells were still swollen and in some places were two or three layers in thickness. Fibroblasts were more prominent than heretofore, and a few giant cells were to be found. Organisms did not occur in any of the walls.

The diaphragm seemed to return to normal even more rapidly than the wall. The serosa was intact and an excess of cells was not present below it, nor were any organisms visible. Polymorphonuclears were not present, and the clasmatocytes were of the small type usually found in normal connective tissue; the fibroblasts were more prominent numerically than in normal tissues.

The peritoneum and the left cavity may be more briefly considered. The conditions found in them are summarized in table 4, which gives the percentages of cells and also the number of animals having infected or sterile cavities at the various intervals.

In the peritoneum, the number of clasmatocytes rose steadily through the twenty-four and forty-eight hour periods, but at seventy-two hours it had returned nearly to the normal proportion. The amount of fluid was never increased, even when the organisms had invaded the cavity, as they had done in two instances. One seems justified in supposing that lack of any acute condition here, even when

infection had occurred, and the steady increase in the proportion of clasmatocytes were the result of the presence of large numbers of these cells in the subjacent tissues and in the omentum.

Microscopically, the peritoneal wall did not show any recognizable changes. The possibility of recognizing such differences is, of course, small, on account of the great area of the wall and the large number of cells present in it.

The course of events in the left cavity was essentially similar to that in the right. The clasmatocytes dropped sharply during the first twenty-four hours and declined slightly further at forty-eight hours. By seventy-two hours, however, they had returned to their normal proportion and the cavities were sterile. The average amounts of fluid paralleled this course of events exactly, increasing and falling with the percentage of polymorphonuclears. Thus the chief difference between the two cavities lay in the speed with which they reacted with a clasmatocytic exudate. As has already been pointed out, in the right cavity the

TABLE 5.—*Duration of Immunity. Study of Animals with a "Peritoneal Focus" That Have Recovered from Initial Infection and Have Been Reinoculated with Varying Doses of Passage Fluid Streptococcus "H" at Intervals Thereafter*

Rabbit	Trypan Blue	Streptococci in Immunizing Dose	Interval	Streptococci in First Test Dose	Result	Interval	Trypan Blue	Streptococci in Second Test Dose	Result
5032	0	2,150	20 days	2,100	Survived	30 days	0	7,250	Survived
5034	0	2,150	20 days	2,100	Survived	30 days	30 cc.	7,250	Survived
5038	0	2,150	30 days	1,200	Survived	30 days	0	2,500	Survived
5035	0	2,150	30 days	1,200*	Survived	30 days	30 cc.	2,500	Survived
5015	0	950†	30 days	1,550	Survived	20 days	30 cc.	2,750	Survived
5016	0	950	30 days	1,550*	Survived	20 days	0	2,750	Survived
5024	0	1,200	20 days	1,550	Survived	20 days	0	2,750	Survived
5025	5 cc.	1,200	20 days	1,550*	Survived	20 days	30 cc.	2,750	Survived

* Inoculation made in left pleural cavity.
† Normal control animal with this dose died in six days.

clasmatocytes reached their low point at twenty-four hours and thereafter increased rapidly in proportion. This difference may be due to the greater stimulus given the right cavity.

Sections of the left cavity taken at twenty-four hours showed a wall somewhat less than twice normal in thickness. Dyed cells were not present; there was a small polymorphonuclear infiltration and a noticeable increase in the monocytes. The diaphragm did not differ essentially from the wall except for the presence in it of dyed cells. At forty-eight hours the mononuclears were more prominent numerically, although the number of cells was always small in comparison with the right cavity. Dyed cells had appeared, and organisms when present were found in and below the serosal cells and undergoing digestion in the subserous mononuclears. At seventy-two hours, the tissue was practically normal: the subserous lymph spaces were enlarged and the serosal cells were slightly swollen. Dyed cells were rarely found, and the bacteria had disappeared.

Of the other organs studied, namely, the spleen, the mesenteric lymph nodes and the right and left superior retrosternal lymph nodes, all were found normal or else had suffered changes not recognizably different from those of the normal infected controls.

The study of animals killed at intervals beyond the three day period showed that the course of events previously indicated continued until an approximately normal condition had been recovered. Thus, the pleural walls and diaphragms of animals killed at five, seven and fourteen days showed tissues in which regressive changes were predominant. That this process is not invariable is demonstrated by the condition of the single animal killed at nine days. Here a chronic infectious condition had set in, and 7 cc. of fluid was present. The tissues surrounding the pleural cavity showed granulating areas of great thickness, that on the diaphragm being more than 1 mm. wide. The cavity had thus been treated much like an abscess and walled off from the rest of the body by granulation tissue. The outcome of an infection of this sort cannot, of course, be determined, although previous experience has shown that animals in which a chronic condition is set up usually recover. Symptoms of infection were not present in the animal when it was killed. A single animal was allowed to survive the infecting dose for fifty days. Histologically, the pleural cavities were normal except for the presence here and there in the subserosa of nests of mononuclears; the peritoneal wall, which showed decided evidence of regressive changes, was still about twice the normal in thickness and contained numerous clasmatocytes.

DURATION OF IMMUNITY

Several of the animals in which a peritoneal focus had been formed and which had survived the intrapleural streptococcic infection were tested for the duration of their immunity. Table 5 gives a summary of this phase of the work. Twenty or thirty days after the dose to test the efficiency of the peritoneal focus, the animals were reinjected intrapleurally with varying amounts of streptococci. In three of them, the reinoculation was in the left cavity. All the animals survived. While some of the doses given were comparatively small, they were still many times the minimum lethal dose (from 80 to 140 times).

After a further interval of twenty or thirty days (i.e., from forty to sixty days after the formation of the peritoneal focus), the animals were again injected with streptococci. This time, however, one of each pair of animals was given 10 cc. of a 1 per cent aqueous solution of trypan blue intraperitoneally each day for the three days preceding the second test dose; 30 cc. of the dye was thus injected. The dye was given to these animals in an effort to ascertain if their immunity could be negatived by a blocking of the clasmatocytes. As the table shows, both the rabbits that received the dye and those that did not were completely protected, even against approximately 450 times the minimum lethal dose.

One should recall here the fact already pointed out that even fifty days after the infection of a peritoneally treated animal a considerable focus of clasmatocyte still remains in its peritoneum. At the same period the pleural surfaces were normal, except for a few small nests of mononuclears. At fourteen days they were also near normal, while the peritoneal focus was still large. Hence, it is probable that at least for a considerable period after its formation the cells of the peritoneal focus

were available in either pleural cavity for the clearing up of infection by streptococci and might have been responsible for the immunity which was present. The failure of the trypan blue to destroy the immunity may have been due to a stimulating effect of the dye on the reticulo-endothelial system, rather than to the depressing effect expected. Thus, Goldzieher and Peck [44] recently showed that the response to infection of the reticulo-endothelial system in vitally stained animals, while less at first, eventually was greater than in unstained controls. The same was found to be true on the injection of toxins. In a recent review of this subject, Gay [45] pointed out that such results are by no means inconsistent with prevention of formation of antibodies or of immunity by dyes, since, as is well known, substances of this sort may have diametrically opposite effects on the body, depending on the amounts in which they are used.

COMMENT

Although the phrase "clasmatocyte transfer" has been used to indicate the process described in the present paper, an assumption has not been made, thereby, that the normal sources of tissue macrophages have failed to supply their usual quota to the irritated area. It must necessarily be true that these places of origin have been stimulated during the formation of the peritoneal focus, since such an enormous increase of clasmatocytes could hardly have come exclusively from cells scattered in the tissues, and since the usual sources have not been interfered with in any way. There are, however, several observations which tend to show that the focus itself is actually the source of many of the cells mobilized in the subsequently irritated area. Histologically, the spleen and mesenteric lymph nodes, from which are derived the monocyte group in general, do not show any more activity in their germinal centers or in their reticulum in animals with a "peritoneal focus" than in animals which merely have an intrapleural irritation. Secondly, in the experiments of Nakahara,[37] a change was not found in the blood picture on the formation of a peritoneal exudate of macrophages; in white cell counts made in this laboratory [46] during experimental transfers between the right and left pleural cavities, material differences could not be detected in the number or proportion of monocytes in the blood, nor could any dyed cells be found in the blood. If cells of the monocyte type were actually being formed in the usual centers for their production in the large numbers found in the exudates, it would seem probable that they would appear in the blood stream in increased numbers. Further-

44. Goldzieher, M. A., and Peck, S. M.: Experimental Studies on Reticulo-Endothelial System, Arch. Path. **3**:629 (April) 1927.

45. Gay, F. P.: Local or Tissue Immunity, Arch. Path. **1**:590 (April) 1926.

46. Unpublished experiments.

more, the appearance in the wall and throughout the diaphragm of large numbers of dyed cells, under the stimulus of a streptococcic infection, points in the same direction, since dyed cells have never been found in the spleen and only irregularly and in small numbers in the mesenteric lymph nodes.

However, when the stimulus is not so great, as in the case of intrapleural irritation by aleuronat-starch, about the same number of dyed cells appear in the pleural walls of animals with a focus as without. This result may be explained on the assumption that the enormous numbers of clasmatocytes in the peritoneum take up the dye to the exclusion of cells in other parts of the body, while in animals without such a focus,

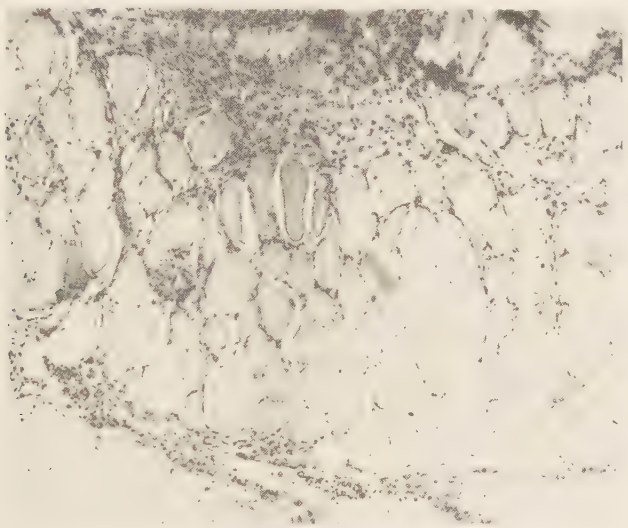

Fig. 2 (rabbit 5042).—Appearance of diaphragm of rabbit with peritoneal focus seventy-two hours after intrapleural streptococcic infection, showing the marked intramuscular invasion of clasmatocytes; × 120.

the dye is free to diffuse away to the rest of the body, including the pleural cavities, and stain the cells there present. Since the aleuronat-starch mixture is not a stimulus comparable to the streptococcus, the number of dyed cells drawn from the focus is only about equal to those stained by the dye in situ. Increased probability is given to this assumption by the observations in groups C and D (table 1). When a focus does not exist (group D), dyed cells are to be found in the pleural exudates and in the walls and diaphragm. When a focus is present, on the other hand (group C), its cells are loaded with dye, while none appears in cells of the pleural exudates and the subjacent tissues are free from it.

Following the vigorous stimulus of streptococci in the pleural cavity, numbers of dyed cells in process of migration may be discovered in the diaphragm (fig. 2). Furthermore, as has already been pointed out, in none of the monocytes in the capillaries has trypan blue ever been found. In this connection an interesting contrast is furnished by the average measurements during the first three days after infection of the peritoneal serosa of the diaphragm. That below the left cavity measures 48 microns, while the average for the portion adjacent to the right cavity is 90 microns; this thickening, as in similar thickenings in the pleurae, is due to the increased numbers of cells, many of which contain dye. Thus there appears a strong tendency for cells in the peritoneum to collect at the point nearest the irritation, preparatory, presumably, to their further migration through the diaphragm.

In this connection, it may be pointed out that the diaphragm is abundantly supplied with lymphatic channels. Küttner [47] stated that both the pleural and peritoneal surfaces are filled with lymph vessels, and MacCallum [48] has pictured the relationship between these lacunae and the mesothelial lining of the pleural and peritoneal cavities. Further, the network of lymph vessels of the peritoneal surface is closely connected with that of the pleural surface. As has already been shown these diaphragmatic lymph spaces become swollen during the pleural infection and in them may be found dye-filled clasmatocytes. Both Cunningham [49] and MacCallum [48] demonstrated that after the intraperitoneal injection of dyes, clasmatocytes loaded with the pigment may be found moving through the peritoneal serosa into the diaphragmatic lymph spaces.

Besides the lymph connections by way of the diaphragm, less direct channels between the two cavities have been demonstrated by various workers. Thus, Tenderloo [50] showed that the mediastinal nodes may be injected from those adjacent to the aorta in the peritoneum, and Franke [51] concluded that there is a direct union between these nodes and those of the lung. Beitzke [52] stated that there is a connection between the two cavities in the vessels which anastomose from the celiac glands to the diaphragmatic pleurae, and Küttner [47] demonstrated that the lymphatic glands of the liver also have a thoracic connection, especially to the left pleural cavity.

SUMMARY

In animals with areas of granulation tissue in the peritoneal wall, intrapleural irritation with aleuronat and starch mixture results in the

47. Küttner, H.: Beitr. z. klin. Chir. **40**:136, 1903.
48. MacCallum, W. G.: Bull. Johns Hopkins Hosp. **14**:105, 1903.
49. Cunningham, R. S.: Am. J. Physiol. **62**:248, 1922.
50. Tenderloo, N. P.: München. med. Wchnschr., 1904, p. 35.
51. Franke, C.: Zey, Beitr. Path., 1912, p. 54.
52. Beitzke, H.: Verhandl. d. deutsch. path. Gesellsch., 1908.

production of an exudate and of a subserous infiltration richer in clasmatocytes than in animals without such a peritoneal focus of cells.

When from 100 to 250 times the minimum lethal dose of a highly virulent strain of hemolytic streptococcus is used intrapleurally in place of the aleuronat and starch mixture, the animals are found to be protected in 73 per cent of the cases. The detailed study of the changes that occur under these circumstances in the pleural wall and pleural exudates strongly suggests that participation of clasmatocytes from the peritoneum is the basis for the intrapleural immunity produced.

Reinoculation of the cavities with multiples of the minimum lethal dose shows immunity in both pleural cavities for at least twenty days and in the right cavity for at least thirty days. The animals are also found immune to a second intrapleural inoculation given from forty to sixty days after the formation of the peritoneal focus.

Vita

Richard Warner Linton, born New Denver, B. C., Canada, Feb. 11, 1899. A.B. Harvard, 1922; M.A. Columbia, 1926.